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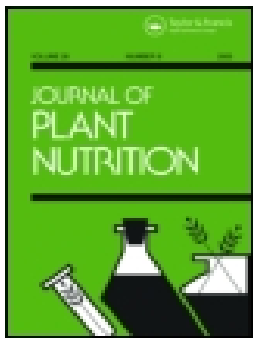
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Micronutrients use efficiency and dry matter yield of annual crops as affected by inorganic and organic amendments

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ABSTRACT

Sustainable crop production is increasing, owing to environmental concerns over the intensive use of chemical fertilizers. This study investigated the effects of equal N substitution by pig slurry compost on micronutrients use efficiency and dry matter yield of *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. Plants were grown in pots with sandy-loam soil and quartz (silica) sand under controlled greenhouse conditions. The treatments were (a) no fertilization (control), (b) NPK fertilizer, (c) pig slurry compost with 50% NPK (PSC), (d) quartz sand with pig slurry compost and 50% NPK (Quartz-PSC) applied at the same rate of 200 mg N kg⁻¹ soil. Inorganic N (NPK) and PSC applications increased seed yield about 10–32%, shoot and root dry matter, on average, by 36% and 21% respectively in mature plants. Micronutrients uptake and use efficiencies were generally lower in PSC- plants than in NPK-plants. Results also showed increased seed Fe (3–28%), Zn (10–26%) and Cu (6–31%) concentrations in NPK and PSC-treated plants. Also, NPK (enriched with Se) significantly elevated seed Se concentration and use efficiency (SeUE) in all plants. Interestingly, PSC addition increased, although not significant, seed Se concentration and SeUE in faba bean and wheat. But no such effect was observed in white lupin. Overall, the results suggest that substitution of half-dose of NPK fertilizer with pig slurry compost is a promising alternative of chemical fertilizer for improved biomass yield and seed micronutrients (Zn, Fe and Cu) concentration in sustainable intensifications.

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Introduction

Trace elements such as zinc (Zn), iron (Fe) a copper (Cu) and selenium (Se) play critical roles in many metabolic and physiological processes and their deficiencies affect plant growth and nutritional quality (Graham et al. 2007; White and Broadley 2009). Plant species vary considerably in their micronutrients uptake mechanisms. Higher plants typically use reduction and/or chelation-based strategies for mobilization and uptake of nutrients in the soil (Graham et al. 2007; Sperotto 2013). For example, dicotyledonous and non-graminaceous monocots use Strategy I (reduction-based strategy) and graminaceous monocots use Strategy II (chelation-based strategy) for Fe uptake (Suzuki et al. 2006; Murata et al. 2015). Micronutrients uptake in plants also involve several high and low affinity specialized transporters for translocation of trace elements to shoot tissues (Sperotto 2013; Cakmak and Kutman 2018). Plant species have different

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Table 1. Selected physical and chemical characteristics of the soil and pig slurry compost (PSC) used in the experiment (dry weight basis).

Characteristics	Soil	PSC
Texture	Sandy-loam	
OM (%)	4.64	10.8
pH (1:2 H ₂ O)	6.15	7.23
pH (CaCl ₂)	6.28	7.41
EC (dSm ⁻¹)	1.34	12.6
Total N (g kg ⁻¹)	13.7	62.7
Available P (g kg ⁻¹)	6.0	17.0
Available K (g kg ⁻¹)	17	48.1
Available Mg (g kg ⁻¹)	154	14.0
Available S (g kg ⁻¹)	242	29.1
Available Na (g kg ⁻¹)	0.13	4.6
Extractable Zn (mg kg ⁻¹)	218	1100
Extractable Fe (mg kg ⁻¹)	163	1420
Extractable Cu (mg kg ⁻¹)	21.9	200
Extractable Mn (mg kg ⁻¹)	78.7	390
Se (mg kg ⁻¹)	–	3.1

micronutrients requirements and the levels in plants depend on the species and developmental stage, as well as, the chemical form and concentration in the soil (White and Broadley 2009; Cakmak and Kutman 2018).

Micronutrient deficiencies in arable soils are widespread and usually caused by long-term soil degradation, as well as, low and unbalanced application of nutrient amendments (Kihara et al. 2016; De Valença et al. 2017). Several studies have shown that prolonged nutrient deficiencies in soils result in reduced crop yields and low micronutrients density in crops (Alloway 2009; Cakmak, Pfeiffer, and McClafferty 2010; Ali et al. 2020). In the last three decades, the use of multinutrient chemical fertilizers (agronomic biofortification) has been successful in addressing nutrient deficiencies in soils and crops. For example, In Finland, a national policy made in 1984 to supplement chemical fertilizers with Se has been very successful and cost-efficient in increasing Se levels of Finnish foods and blood Se levels of the population (Eurola et al. 1990; Alfthan et al. 2015). Other studies have also shown that soil and foliar applications of multinutrient chemical fertilizers can increase yields and nutrient contents in food crops and feed (Eurola et al. 1990; Cakmak 2008; White and Broadley 2009; Cakmak and Kutman 2018). Although multinutrient chemical fertilizers are central to agronomic biofortification of crops with micronutrients, there is renewed interest in using less chemical fertilizers in sustainable food production.

The use of organic nutrient resources including animal manures, slurries and composts represent a sustainable alternative to chemical fertilizers in sustainable intensifications (Anwar et al. 2018; Kizito et al. 2019). Several studies have shown that using organic amendments as fertilizers to meet crops N requirements can also enhance soil (physical, chemical and biological) characteristics over long period of time (Edmeades 2003; Jindo et al. 2016; Sileshi et al. 2019; Gopalakrishnan et al. 2020). Animal manures and slurries contain readily available and organically-bound plant nutrients which serve as renewable source of nutrients especially nitrogen (N) and phosphorus (P) to plants (Edmeades 2003; Antolín-Rodríguez et al. 2020). Manures can also be an important source of trace elements (Fe, Zn, Cu, Se, B, Mo, Mn, and Cl) for crops (Jensen 2013; Provolto et al. 2018). However, long-term land applications of animal manures and slurries may result in the excessive accumulation of salts, heavy metals and pathogens in arable soils (Berenguer et al. 2008; Provolto et al. 2018).

Evidence of the positive effects of manure and other organic amendments on crop yields and nutrients (N, P, K) uptake is increasing (Anwar et al. 2018; Antolín-Rodríguez et al. 2020; Sileshi et al. 2019; Ali et al. 2020). However, comprehensive data on micronutrients recycling and bio-availability in the manure-soil-crop system, and how well they meet crops, humans and animal requirements are lacking. The aims of this study were to examine, under controlled conditions,

the effect of equal N substitution by pig manure compost on micronutrients use efficiency (MUE) and dry matter yield of *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. In addition, N and P interactions with trace elements (Fe, Zn, Cu and Se) were also investigated.

Materials and methods

Pig slurry compost (PSC)

Pig slurry (mixture of urine, feces and waste water) used in this study was obtained from fattening pigs in a commercial piggery located in Helsinki (Finland). Pig slurry was collected in plastic containers and stored at 5°C. To improve its fertilizer efficiency and also kill pathogens, pig slurry (C/N ratio of 10:1) was mixed with wheat straw (C/N ratio of 105:1) in the ratio of 3:1 and decomposed for eight (8) weeks. The mixture was turned weekly to maintain porosity. Subsamples of the compost were ground and sieved (<2.00 mm) and taken for analysis at Viljavuuspalvelu Oy, Mikkeli, Finland. The dry matter of the compost was 55%. Selected physical-chemical properties of the pig slurry compost are described in Table 1.

Pot experiment

Pot experiment with sandy-loam soil (76% sand, 19% silt and 5% clay with 4.6% organic C) was conducted in the glasshouse at the Department of Agricultural Sciences, University of Helsinki, Finland (60°13'38" N, 25°10'00" E). Subsamples of the air-dried and sieved (1.75 mm) soil were stored at -20°C for further analysis. Selected physical and chemical properties of the soil are described in Table 1. The experiment was carried out in a randomized complete block design in four replicates. The main treatments were (a) no fertilization (control), (b) mineral fertilizer (NPK) containing Na₂SeO₄ (0.0025 mg Se kg⁻¹), (c) pig slurry compost with 50% NPK (PSC), (d) quartz sand with pig slurry compost and 50% NPK (Quartz-PSC). Commercial quartz (silica) sand (grain size 0.1-0.6 mm, SP-Minerals Oy, Nilsian kvartsi, Finland) was used in this study to estimate the adsorption of nutrients onto soil particles.

Polyethene-lined 3 L plastic pots were filled with 2 kg of sieved (1.75 mm) and thoroughly mixed with sandy-loam soil and quartz sand. Mineral fertilizer (N-P-K: 20-9-12, Yara Mila NK2 with Single Super Phosphate, Yara Suomi Ltd. Finland) and pig slurry compost with 50% NPK (PSC) were applied at the same rate of 200 mg N kg⁻¹ soil. The moisture content of the soil was adjusted to 70% of water-holding capacity. The pots were then incubated at room temperature for four weeks and water losses exceeding 10% during the incubation period was compensated by addition of distilled water.

Faba bean (*Vicia faba* L. cv. Alexia), white lupin (*Lupinus albus* L. cv. Vesna) and spring wheat (*Triticum aestivum* L. cv. Quarna) seeds were sown (10 seeds per pot) on September 9, 2015. Faba bean and white lupin seeds were not inoculated with *rhizobium* spp. Plants were later thinned to three plants per pot on day 7 after germination. The pots were re-arranged weekly and growing conditions in the glasshouse were maintained at 25/18 °C (day/night) temperature, relative humidity (RH) of 60–80% and a photoperiod of 14/10 h (illumination/darkness). Natural light in the glasshouse was supplemented with 400 W high-pressure sodium lamps (Lucalox, LU 400/HO/T/40NG, Hungary) at a mean photosynthetic photon flux density (PPFD) of 450 μmol photons m⁻² s⁻¹. Conditions were continuously monitored throughout the experiment.

To estimate the uptake and accumulation of micronutrients in plant tissues during growth, each species was harvested twice: (a) stem elongation stage (GS 39) (7 weeks after germination) and (b) maturity or ripening stage (GS 87) (13 weeks after germination) according to the BBCH scale (Meier 1997), hereafter referred to as H1 and H2 respectively. At each harvest, shoots and roots were separated and the roots were carefully and thoroughly rinsed with deionized water and blotted dry with tissue. The plant samples were dried at 70 °C for at least 48 h to a constant

weight, and the dry weights (DW) recorded. Subsamples of harvested materials were milled to fine particle size of 1 mm with a cutting mill (Retsch ZM 200, Germany) with titanium knife to avoid micronutrient contamination. Subsamples (0.3 g) were analyzed for total N and C contents using the Dumas combustion method (Hansen 1989) using a Vario Max CN analyzer (Elementar GmbH, Germany). Total C and N were expressed as percentage weight of sample.

Soil analyses

Soil texture was determined by mechanical analysis using the pipette method (Gee and Bauder 1986). The soil material was dispersed in soil-water suspension using sodium hexametaphosphate, followed by fractionation and quantification of each particle-size interval by sedimentation. Soil cation-exchange capacity (CEC) was determined by the ammonium-saturation and distillation method using a buffered 1 M $\text{CH}_3\text{COO-NH}_4$ at pH 7 and unbuffered 1 M KCl solutions (Schollenbergen and Simon 1945).

Soil pH and electrical conductivity were measured in deionized water and in 0.01 M CaCl_2 (1:2.5w/v) (Sumner 1994). The suspensions were mixed thoroughly, covered with parafilm and incubated overnight at room temperature, where after pH was measured using a glass electrode (Schott Instrument TM). The electrical conductivity of the solution phase was measured using the electrical conductivity meter (JENWAY 4020).

Subsamples of the soil were taken for further analysis of plant-available nutrients at Viljavuusalvelu Oy, Mikkeli, Finland using ICP-OES (inductively coupled plasma optical emission spectrometry).

Plant analyses

Macro- and micro nutrients (Ca, Mg, K, P, S, Fe, Zn, Cu, Mn, B, Cr, Ni, Cd, Al, Pb and As) concentrations in plant samples were determined using the microwave digestion method and analyzed using ICP-OES (iCAP 6200, Thermo Fisher Scientific Inc., Cambridge, UK). Duplicate 250 mg of milled plant samples were weighed into acid-washed Teflon digestion tubes and mixed with 6 mL of concentrated nitric acid (HNO_3) (ICP/AAS grade) and 1 mL of hydrogen peroxide (H_2O_2) (analytical grade). The mixture was incubated overnight in a fume-hood at room temperature. The mixture was then digested in a closed-vessel microwave digester (MAR SXpress, MARS 240/50, CEM, Mathews, NC, USA) at stepwise increased temperature. After digestion, samples were filtered through ashless filter paper (Whatman paper, GE Healthcare Companies, UK) into 50 mL volumetric flasks and filled to the mark with deionized water (Millipore Milli Q/Q-POD TM, Ireland). The samples were immediately stored at -20°C for the elemental analysis by ICP-OES. All glasswares were acid-washed to prevent contamination. Accuracy of the measurements was verified using in-house reference sample (meal) for every batch of elemental analysis. The micro-elements concentration was expressed in mg kg^{-1} (DM).

For the determination of Se, subsamples (0.2–0.5 g) of dried seeds or grains were weighed into wet digestion tubes. The samples were incubated overnight at room temperature in 10 mL of a concentrated acid mixture ($\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$ in a ratio of 2.5:1.5:1). Selenium was extracted using ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone (APDC-MIBK) as described by Keskinen, Turakainen, and Hartikainen (2010) and analyzed in duplicate by ICP-OES (iCAP 6000 Series, Thermo Scientific). Three in-house samples were used as standards. Selenium concentration was expressed in $\mu\text{g g}^{-1}$ (DW).

Table 2. Dry weights (DW) of shoots (leaves and stems), roots, seed pods and seeds of *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. treated with inorganic fertilizer (NPK) and pig slurry compost with 50% NPK (PSC) at 200 mg N kg⁻¹ soil and sampled at stem elongation (H1) and seed ripening (H2) stages.

Treatment	Shoot		Root		Seed pod (g pot ⁻¹)	Seed (g pot ⁻¹)
	H1 (g pot ⁻¹)	H2 (g pot ⁻¹)	H1 (g pot ⁻¹)	H2 (g pot ⁻¹)		
Faba bean						
Control	4.3 ± 0.1 ^c	9.3 ± 0.4 ^c	1.7 ± 0.1 ^b	3.2 ± 0.3 ^b	0.2 ± 0.0 ^c	1.5 ± 0.0 ^b
NPK	9.2 ± 0.5 ^a	14.2 ± 0.4 ^a	3.3 ± 0.2 ^a	4.5 ± 0.0 ^a	0.5 ± 0.1 ^b	2.1 ± 0.0 ^a
PSC	5.4 ± 0.6 ^b	11.7 ± 0.9 ^b	2.0 ± 0.2 ^b	3.1 ± 0.1 ^b	0.9 ± 0.0 ^a	2.0 ± 0.0 ^a
Quartz-PSC	4.0 ± 0.2 ^c	7.9 ± 0.6 ^d	2.0 ± 0.1 ^b	2.2 ± 0.4 ^c	–	–
White lupin						
Control	3.4 ± 0.1 ^c	5.8 ± 0.3 ^c	1.0 ± 0.1 ^c	2.6 ± 0.1 ^b	0.6 ± 0.0 ^a	1.4 ± 0.0 ^c
NPK	4.3 ± 0.4 ^b	7.7 ± 0.2 ^b	1.8 ± 0.0 ^b	4.5 ± 0.4 ^a	0.7 ± 0.1 ^a	1.8 ± 0.1 ^a
PSC	7.5 ± 0.2 ^a	12.2 ± 1.0 ^a	2.6 ± 0.3 ^a	4.8 ± 0.6 ^a	0.9 ± 0.1 ^a	1.7 ± 0.0 ^b
Quartz-PSC	2.0 ± 0.1 ^d	3.2 ± 0.1 ^d	0.5 ± 0.0 ^d	0.5 ± 0.1 ^c	–	–
Wheat						
Control	1.5 ± 0.1 ^d	2.5 ± 0.1 ^c	0.5 ± 0.0 ^c	1.3 ± 0.0 ^c	0.4 ± 0.1 ^c	1.8 ± 0.0 ^c
NPK	9.6 ± 0.4 ^b	15.6 ± 0.5 ^b	1.8 ± 0.1 ^b	6.6 ± 0.8 ^a	1.0 ± 0.1 ^b	2.0 ± 0.0 ^b
PSC	14.7 ± 0.8 ^a	20.5 ± 0.7 ^a	2.4 ± 0.1 ^a	6.9 ± 0.6 ^a	1.5 ± 0.2 ^a	2.2 ± 0.0 ^a
Quartz-PSC	8.0 ± 0.8 ^c	16.3 ± 1.0 ^b	1.7 ± 0.1 ^b	4.2 ± 0.5 ^b	0.8 ± 0.0 ^b	2.0 ± 0.0 ^b

Data are means ± SE; n = 3–4. Values within a column with different letters (in each plant species) are significantly different at $p \leq 0.05$.

Nutrient efficiency indices

Nutrient efficiency indices were calculated for trace elements Zn, Fe, Cu and Se using indices, where applicable, by Moll, Kamprath, and Jackson (1982) and Baligar, Fageria, and He (2001). *Nutrient content* (mg kg⁻¹ DM) was estimated by multiplying the concentration of nutrients in plant tissues by dry matter yield. *Micronutrient uptake efficiency* (MU_PE, mg/mg) in plants was calculated by dividing the total aboveground nutrient content (seed or grain and shoot or straw) by the total available nutrients from soil material, fertilizer and slurry (Moll, Kamprath, and Jackson 1982). *Micronutrient utilization efficiency* (MU_TE, mg/mg) was calculated by dividing seed or grain yield by the total aboveground nutrient content (seed or grain and shoot or straw). *Micronutrient use efficiency* (MUE, mg/mg) was defined as seed or grain yield produced per unit of available nutrients. It was calculated by dividing seed or grain yield by the total available nutrients from soil material, fertilizer and slurry (Moll, Kamprath, and Jackson 1982; Baligar, Fageria, and He 2001).

Statistical analyses

The effects of inorganic N (NPK) and pig slurry compost (PSC) fertilization on above-ground yield, micronutrients uptake and use efficiency were tested statistically using SPSS (version 22) (IBM SPSS Statistics, Version 22.0. Armonk, NY, USA). Data was subjected to two-way ANOVA and differences in means compared with Tukey's test at $p \leq 0.05$. Correlation analysis was performed on seed macro- and micronutrients concentration in faba bean, white lupin and wheat separately.

Results

Biomass, seed yield and N concentration

Inorganic N (NPK) and pig slurry compost (PSC) applications significantly increased shoot and root biomass of plants at the earlier growth stage (H1) and in mature plants (H2) (Table 2). Interestingly, in NPK- treated plants, shoot and root biomass was highest in faba bean in all harvests. However, in PSC-treated plants, shoot and root biomass production was most significant in white lupin and wheat plants (Table 2). Addition of NPK and PSC also increased seed yield about 12% in wheat, and 22 to 32% in faba bean and white lupin (Table 2). Furthermore, NPK

Table 3. Mean selenium (Se) concentration ($\mu\text{g g}^{-1}$ DW) and use efficiency (SeUE) ($\mu\text{g } \mu\text{g}^{-1}$) in the shoots and seeds of *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. treated with inorganic fertilizer (NPK) and pig slurry compost with 50% NPK (PSC) at 200 mg N kg^{-1} soil and sampled at stem elongation (H1) and seed ripening (H2) stages.

	Shoot Se ($\mu\text{g g}^{-1}$ DW)		Seed Se ($\mu\text{g g}^{-1}$ DW)	SeUE ($\mu\text{g } \mu\text{g}^{-1}$)
Treatment	H1	H2		
Faba bean				
Control	0.01 ^c	0.01 ^b	0.01 ^b	–
NPK	0.25 ^a	0.13 ^a	0.29 ^a	35.21 ^a
PSC	0.12 ^b	0.05 ^b	0.11 ^b	31.97 ^b
Quartz-PSC	NS	NS	NS	NS
SE	0.04	0.02	0.03	1.05
White lupin				
Control	0.01 ^c	0.02 ^b	0.01 ^b	–
NPK	0.22 ^a	0.15 ^a	0.18 ^a	29.85 ^a
PSC	0.14 ^b	0.07 ^b	0.08 ^b	27.58 ^b
Quartz-PSC	NS	NS	NS	NS
SE	0.01	0.01	0.02	0.57
Wheat				
Control	0.03 ^c	0.01 ^c	0.01 ^c	–
NPK	0.31 ^a	0.17 ^a	0.28 ^a	32.88 ^b
PSC	0.19 ^b	0.08 ^b	0.13 ^b	35.99 ^a
Quartz-PSC	0.14 ^b	0.05 ^b	0.12 ^b	32.79 ^b
SE	0.04	0.02	0.03	1.15

Data are means; n=8. Values within a column with different letters (in each plant species) are significantly different at $p \leq 0.05$.

*NS- no seeds produced

and PSC applications exerted positive effect on N and C concentrations, C/N ratio and crude protein content in the seeds (data not shown). Treatment with quartz sand and pig slurry compost (Quartz-PSC) was included to reveal the potential retention of applied micronutrients in soil particles. Faba bean and white lupin plants did not produce seeds in quartz sand (Table 2). Therefore, only the results on wheat in this treatment are discussed later.

Selenium (Se) concentration in shoots

The effect of Se-enriched NPK and PSC on Se concentration in shoots (leaves and stems) changed with growth of plants (Table 3). At the earlier growth stage (H1), in NPK-plants, shoot Se concentration was high in wheat ($0.31 \mu\text{g Se g}^{-1}$ DW) as compared with faba bean ($0.25 \mu\text{g Se g}^{-1}$ DW) and white lupin ($0.22 \mu\text{g Se g}^{-1}$ DW). Although PSC-induced changes in Se concentrations in shoot tissues was generally low than those in NPK-plants, shoot Se concentration increased about (6-14 fold) compared with control plants. In all treatments, Se concentration in plants shoots decreased in mature plants (H2) (Table 3). Se concentration in root tissues was not analyzed due to lack of plant material. Therefore, only the results in shoot tissues are discussed later.

Selenium (Se) concentration in seeds

As expected, NPK application exerted positive effect on seed Se concentration in plants (Table 3). Selenium (Se) content (concentration x dry weight) was high in faba bean and wheat ($0.28 \mu\text{g g}^{-1}$ DW) and relatively low in white lupin ($0.18 \mu\text{g g}^{-1}$ DW). On the other hand, the effect of PSC on seed Se content was inconsistent (Table 3). Although in faba bean and white lupin there were no significant increases in Se concentration, PSC markedly elevated Se concentration ($0.13 \mu\text{g g}^{-1}$ DM) in wheat grains (Table 3). Pig slurry compost with half-dose of NPK fertilizer (PSC) contained $61.85 \mu\text{g g}^{-1}$ Se per pot (Table 1), but Se use efficiency (SeUE) was relatively low

Table 4. Seed micronutrients concentration (mg kg⁻¹ DW) of *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. treated with inorganic fertilizer (NPK) and pig slurry compost with 50% NPK (PSC) at 200 mg N kg⁻¹ soil and sampled at maturity (H2) (13 weeks after germination).

Treatment	Fe (mg kg ⁻¹ DW)	Zn (mg kg ⁻¹ DW)	Cu (mg kg ⁻¹ DW)
Faba bean			
Control	46.5 ± 3.3 ^a	33.4 ± 0.7 ^a	5.0 ± 0.1 ^a
NPK	52.7 ± 1.1 ^b	43.8 ± 1.3 ^b	6.0 ± 0.1 ^b
PSC	64.0 ± 0.4 ^c	37.2 ± 1.1 ^c	5.6 ± 0.0 ^c
Quartz-PSC	NS	NS	NS
White lupin			
Control	53.5 ± 0.2 ^a	33.2 ± 0.1 ^a	5.1 ± 0.1 ^a
NPK	60.13 ± 2.2 ^b	39.2 ± 1.3 ^b	6.2 ± 0.2 ^b
PSC	74.4 ± 0.6 ^c	36.6 ± 0.5 ^c	5.7 ± 0.1 ^c
Quartz-PSC	NS	NS	NS
Wheat			
Control	75.8 ± 0.8 ^a	22.4 ± 1.1 ^a	5.7 ± 0.1 ^a
NPK	78.5 ± 0.8 ^b	30.3 ± 0.4 ^b	6.1 ± 0.2 ^b
PSC	104.7 ± 0.2 ^c	26.0 ± 0.2 ^c	8.2 ± 0.0 ^c
Quartz-PSC	99.5 ± 1.8 ^c	24.9 ± 0.6 ^a	7.8 ± 0.2 ^c

Data are means ± SE; n = 3-4. Values within a column with different letters (in each plant species) are significantly different at $p \leq 0.05$.

*NS- no seeds produced

Table 5. Mean micronutrients concentration (mg kg⁻¹ DW) of leaf, stem and root of *Vicia faba* L. and *Lupinus albus* L. treated with inorganic fertilizer (NPK) and pig slurry compost with 50% NPK (PSC) at 200 mg N kg⁻¹ soil and sampled at stem elongation (H1) and seed ripening (H2) stages.

Treatment	Faba bean						White lupin					
	Leaf		Stem		Root		Leaf		Stem		Root	
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2
Fe (mg kg⁻¹ DW)												
Control	68.3 ^b	59.2 ^b	22.7 ^b	19.5 ^a	7.2 ^c	4.0 ^b	69.7 ^c	61.6 ^b	12.2 ^c	8.4 ^c	4.4 ^c	3.6 ^c
NPK	70.6 ^b	62.5 ^b	23.7 ^b	21.1 ^a	7.0 ^c	3.9 ^b	73.5 ^c	65.6 ^b	13.6 ^c	10.3 ^c	6.3 ^c	2.8 ^c
PSC	92.7 ^a	69.6 ^a	25.0 ^a	22.5 ^a	9.5 ^b	18.8 ^a	123.5 ^a	79.5 ^a	30.3 ^a	37.6 ^a	15.3 ^a	17.6 ^a
Quartz-PSC	85.2 ^a	72.6 ^a	17.6 ^c	13.8 ^a	13.5 ^a	16.4 ^a	90.7 ^b	81.2 ^a	14.6 ^b	28.0 ^b	10.1 ^b	13.2 ^b
SE	9.3	4.8	3.3	2.4	1.4	3.3	17.8	12.4	2.6	5.9	3.5	5.8
Zn (mg kg⁻¹ DW)												
Control	30.4 ^c	23.1 ^d	10.8 ^c	8.7 ^c	6.3 ^b	5.2 ^c	34.3 ^c	30.3 ^c	11.2 ^d	8.1 ^d	6.4 ^c	3.2 ^c
NPK	38.4 ^b	24.7 ^c	14.2 ^b	9.5 ^b	7.0 ^b	5.8 ^c	41.5 ^b	33.3 ^b	12.3 ^c	10.4 ^c	6.4 ^c	3.9 ^b
PSC	45.4 ^a	36.0 ^a	17.7 ^a	12.7 ^a	14.9 ^a	12.1 ^a	46.1 ^a	39.2 ^a	21.5 ^a	13.3 ^b	13.9 ^a	8.0 ^a
Quartz-PSC	47.0 ^a	33.2 ^b	14.9 ^b	10.4 ^b	15.5 ^a	8.1 ^b	48.4 ^a	37.3 ^a	15.9 ^b	17.2 ^a	7.9 ^b	2.3 ^d
SE	4.1	2.7	3.5	1.9	2.3	1.6	5.9	6.3	2.7	1.9	1.2	0.7
Cu (mg kg⁻¹ DW)												
Control	7.0 ^c	5.3 ^b	2.3 ^a	1.6 ^b	2.0 ^c	1.5 ^c	6.4 ^c	4.5 ^c	2.0 ^c	1.8 ^b	1.6 ^b	1.1 ^c
NPK	7.9 ^c	5.7 ^b	2.4 ^a	1.7 ^b	2.3 ^c	1.8 ^c	7.0 ^c	6.0 ^c	2.1 ^c	2.1 ^b	2.2 ^b	1.4 ^c
PSC	13.3 ^a	9.9 ^a	2.4 ^a	3.0 ^a	4.2 ^a	5.5 ^b	18.4 ^a	13.2 ^a	6.3 ^a	3.9 ^a	7.4 ^a	10.4 ^a
Quartz-PSC	11.5 ^b	10.3 ^a	1.5 ^b	1.4 ^b	3.8 ^b	6.6 ^a	15.8 ^b	10.1 ^b	5.1 ^b	3.6 ^a	6.5 ^a	2.6 ^b
SE	1.5	0.6	0.3	0.4	0.3	0.2	1.6	2.2	0.4	0.6	0.7	0.3

Data are means; n = 4. Values within a column with different letters (in each trace element) are significantly different at $p \leq 0.05$.

in faba bean and white lupin plants (27.58–31.97 µg/µg) compared with NPK plants (29.85–35.21 µg/µg) (Table 3).

Micronutrients (Fe, Zn and Cu) concentration in shoots and roots

The effect of NPK and PSC on micronutrients uptake and accumulation changed with growth of plants (Tables 4 and 5). At the earlier growth stage (H1), NPK application exerted positive effect on Fe, Zn and Cu concentrations in shoots (leaves and stems) and roots of all plants

Table 6. Mean micronutrients concentration (mg kg⁻¹ DW) of shoot and root of *Triticum aestivum* L. treated with inorganic fertilizer (NPK) and pig slurry compost with 50% NPK (PSC) at 200 mg N kg⁻¹ soil and sampled at stem elongation (H1) and seed ripening (H2) stages.

Treatment	Fe (mg kg ⁻¹ DW)				Zn (mg kg ⁻¹ DW)				Cu (mg kg ⁻¹ DW)			
	Shoot		Root		Shoot		Root		Shoot		Root	
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2
Control	101.1 ^c	80.6 ^c	9.6 ^c	6.5 ^c	31.5 ^c	28.1 ^c	4.6 ^c	5.1 ^b	8.3 ^c	6.2 ^c	2.0 ^c	6.0 ^b
NPK	107.3 ^c	84.8 ^c	7.5 ^c	5.0 ^c	37.5 ^b	29.4 ^b	5.5 ^c	6.0 ^b	8.7 ^c	6.6 ^c	1.5 ^c	2.1 ^c
PSC	158.1 ^b	125.8 ^a	22.1 ^a	13.0 ^b	45.8 ^a	35.0 ^a	8.1 ^a	12.5 ^a	12.8 ^b	16.7 ^b	10.3 ^a	14.2 ^a
Quartz-PSC	167.4 ^a	104.8 ^b	14.8 ^b	18.4 ^a	46.8 ^a	35.2 ^a	6.8 ^b	13.5 ^a	17.6 ^a	19.9 ^a	7.7 ^b	15.9 ^a
SE	18.9	12.3	3.6	3.2	4.6	2.9	0.5	1.8	2.3	1.1	0.4	0.3

Data are means; n=4. Values within a column with different letters are significantly different at $p \leq 0.05$. Columns are tested separately.

Table 7. Pearson correlation coefficients among seed macronutrients (N, P) and trace elements (Zn, Fe, Zn, Cu and Se) in *Vicia faba* L. (above the diagonal), *Lupinus albus* L. (below the diagonal) and *Triticum aestivum* L.

Nutrient	N	P	Fe	Zn	Cu	Se
N	1	0.760**	0.857**	0.413	0.627*	0.399
P	0.804**	1	0.590	0.872**	0.960**	0.860**
Fe	0.946**	0.752**	1	0.267	0.516	0.146
Zn	0.514	0.471	0.441	1	0.927**	0.904**
Cu	0.512	0.457	0.410	0.988**	1	0.852**
Se	0.130	0.251	-0.088	0.728**	0.761**	1
Wheat	N	P	Fe	Zn	Cu	Se
N	1					
P	0.708**	1				
Fe	0.720**	0.768**	1			
Zn	0.907**	0.575*	-0.130	1		
Cu	0.209	0.793**	0.991**	0.280	1	
Se	0.925**	0.861**	0.449	0.829**	0.488	1

*:bold numbers mean significant differences.

*, **, ***:significant differences at $P \leq 0.05$, 0.01 and 0.001 respectively.

as compared with the control (Tables 4 and 5). However, PSC-induced changes in Fe, Zn and Cu concentrations in plant tissues were more pronounced than those in NPK. At the earlier growth stage (H1), PSC markedly increased shoot (leaves and stems) Fe about (23- 47%), Zn about (31-35%), and Cu about (35- 66%) in all plants as compared with the control. But, the concentrations tended to decrease in mature plants (H2). However, in roots, micronutrients (Fe, Zn and Cu) accumulations were markedly high at the later growth stage (H2) in PSC-plants (Tables 4 and 5). Although trace elements concentration in PSC was high (Table 1), micronutrients uptake (UpE) and utilization (UtE) efficiency values for Fe, Zn and Cu were generally lower in PSC-plants than in NPK-plants (Table 6). The concentrations of Cr, Pb, Al, As and Ni in all analyzed plant samples were below detection limit in all plant samples.

Micronutrients (Fe, Zn and Cu) concentration in seeds

Inorganic N (NPK) and PSC applications exerted positive effects on seed micronutrients concentration in faba bean, white lupin and wheat (Table 7). On the average, NPK and PSC increased seed Fe content (concentration x dry weight) about 3-28% in all plants. There were also significant increases in seed Zn content in NPK-plants (15-26%) and PSC-plants (9-14%) as compared with control plants (Table 7). Similar observations were made in seed Cu content. However, the PSC-induced changes were less profound in faba bean and white lupin (Table 7). Although Cu concentration in shoot tissues was markedly elevated in all plants

Table 8. Mean micronutrients uptake (MUpE), utilization (MUE) and use (MUE) efficiencies of *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. treated with inorganic fertilizer (NPK) and pig slurry compost with 50% NPK (PSC) at 200 mg N kg⁻¹ soil.

Treatment	Faba bean			White lupin			Wheat		
	MUpE (mg/mg)	MUE (mg/mg)	MUE (mg/mg)	MUpE (mg/mg)	MUE (mg/mg)	MUE (mg/mg)	MUpE (mg/mg)	MUE (mg/mg)	MUE (mg/mg)
Fe									
Control	60.0 ^b	6.9 ^c	415.4 ^b	57.2 ^b	6.5 ^b	373.1 ^b	71.9 ^a	6.9 ^b	494.9 ^b
NPK	64.1 ^a	9.2 ^a	590.9 ^a	62.0 ^a	8.1 ^a	500.9 ^a	75.5 ^a	7.3 ^a	551.9 ^a
PSC	35.6 ^c	7.2 ^b	253.8 ^c	44.6 ^c	4.9 ^c	216.1 ^c	50.2 ^c	5.7 ^c	284.3 ^c
Quartz-PSC	24.2 ^d	NS	NS	27.4 ^d	NS	NS	47.9 ^b	5.5 ^c	260.3 ^c
SE	4.4	0.3	41.8	3.6	0.4	35.6	3.3	0.2	33.0
Zn									
Control	49.3 ^a	14.0 ^c	691.7 ^a	54.3 ^a	11.5 ^b	621.3 ^a	38.2 ^a	21.6 ^a	824.1 ^a
NPK	31.2 ^b	16.2 ^a	505.9 ^b	32.6 ^b	13.2 ^a	428.8 ^b	23.2 ^b	20.4 ^a	472.5 ^b
PSC	23.0 ^c	13.2 ^b	300.7 ^c	24.0 ^c	10.8 ^b	256.0 ^c	16.4 ^c	20.6 ^b	336.7 ^c
Quartz-PSC	15.9 ^d	NS	NS	18.0 ^b	NS	NS	16.1 ^c	19.0 ^b	308.3 ^c
SE	3.2	0.5	48.5	3.6	0.4	45.2	2.3	0.5	53.0
Cu									
Control	9.7 ^b	70.9 ^a	688.6 ^b	9.1 ^b	68.7 ^b	618.5 ^b	9.3 ^a	88.6 ^b	820.3 ^b
NPK	11.0 ^a	89.3 ^a	979.6 ^a	10.8 ^a	76.9 ^a	830.3 ^a	9.9 ^a	92.9 ^a	914.8 ^a
PSC	6.4 ^c	56.9 ^a	363.3 ^c	8.9 ^c	35.4 ^c	309.4 ^c	7.0 ^c	58.1 ^c	406.9 ^c
Quartz-PSC	4.5 ^d	NS	NS	6.3 ^d	NS	NS	8.4 ^b	44.8 ^d	372.6 ^c
SE	0.7	4.2	76.3	0.5	5.7	65.1	0.3	5.4	62.4

Data are means; n=4. Values within a column with different letters (in each trace element) are significantly different at $p \leq 0.05$.

(Tables 4 and 5), PSC slightly increased seed Cu concentration about 10% (Table 7). However, in wheat plants, PSC increased grain Cu concentration by 44% as compared with the control. Interestingly, Cu use efficiency (CuUE) values in PSC-plants were much lower compared to NPK and control plants (Table 6).

Correlation between N, P and micronutrients concentration in seeds

Correlation analysis showed strong positive correlation between seed macro-and micronutrients in plants (Table 8). Our results showed significant ($P < 0.01$) and positive correlation coefficients for seed concentration of N and Fe ($r = 0.887$, $r = 0.946$) in faba bean and white lupin respectively. There was positive but non-significant correlation between N and Zn in these plants. Furthermore, P concentration in seeds also had significant and positive correlation coefficients for Zn ($r = 0.872$), Cu ($r = 0.627$) and Se ($r = 0.860$, $P < 0.01$) in faba bean, as well as, Fe ($r = 0.752$) in white lupin (Table 8). Similarly, in wheat, grain N and P concentrations had positive effects on Fe, Zn and Cu. Further analysis also showed significant correlation for N-Se ($r = 0.925$, $P < 0.01$) and P-Se ($r = 0.861$, $P < 0.01$) in wheat (Table 8).

Discussion

The application of mineral fertilizer (NPK) and pig slurry compost with half dose of NPK (PSC) exerted positive effects on plant growth and development. Even though, pig slurry compost used in this study contained high salt content (Table 1), the results show no visible indication that growth and other physiological processes in plants were impaired. Earlier studies have shown that, application of animal manures and slurries increase soil salinity and the risk of secondary salinity. But, the severity depends on the duration of application, soil and manure characteristics (Awad, Solaimanfathy, and El-Nakhlawy 2015; Leogrande and Vitti 2019). In the present study, NPK and PSC additions increased seed yield, shoot and root dry matter accumulation (Table 2). This is consistent with the findings of Sharma and Subehia (2003) in wheat, and Santos et al.

(2018) in annual ryegrass (*Lolium multiflorum* Lam.). These authors reported of significant increase in seed yield and shoot biomass after NPK and swine manure applications. The increase in aboveground dry matter yield in this study, may be attributable to increased N use efficiency with stimulatory effects on photosynthesis (Makino 2011), carbohydrate and protein metabolism in plants (Welch and Graham 2000; Graham et al. 2007). The reasons for the lack of seed formation in mature faba bean and white lupin plants grown in quartz sand, in this study, thus, remain unclear. However, it is possible that quartz sand may have impaired growth in these plants, owing to decreases in root and shoot biomass (Table 2).

In the present study, NPK and PSC additions exerted positive effects on micronutrients (Zn, Fe and Cu) uptake and accumulation in the shoots and roots of plants (Tables 4 and 5). At the earlier growth stage (H1), micronutrients accumulation was more pronounced in the shoots than roots. But, the levels in the shoots and roots tended to decrease with plant growth (H2) (Tables 4 and 5). This may be attributable to biomass dilution due to increased shoot biomass and remobilization of micronutrients from leaves into seeds (Graham and Stangoulis 2003; Cakmak and Kutman 2018). Earlier studies have reported that, the uptake of organically-bound trace elements is low and their translocation from roots to vegetative parts can be inefficient (Berenguer et al. 2008; Nikoli and Matsi 2011). The high shoot: root micronutrients (Fe, Zn and Cu) concentration in PSC-plants in this study (Tables 4 and 5) may be attributable to increased mineralization of organic-bound trace elements into plant-available forms due to the longer decomposition and incubation time before seed sowing.

The addition of Se-enriched NPK markedly elevated seed Se concentration ($0.18\text{--}0.29\ \mu\text{g g}^{-1}$ DW) and Se use efficiency (SeUE) ($29.85\text{--}35.21\ \mu\text{g}/\mu\text{g}$) in plants (Table 3). In contrast, seed Se concentration ($0.05\text{--}0.13\ \mu\text{g g}^{-1}$ DW) and SeUE ($27.58\text{--}35.99\ \mu\text{g}/\mu\text{g}$) were relatively low in PSC-plants (Table 3). Even though earlier studies on organic Se uptake and the translocation to harvestable parts in oilseed rape (*Brassica napus* L.) (Ebrahimi et al. 2015), wheat and oats (*Avena sativa* L.) (Eich-Greatorex et al. 2007) suggest organic Se translocation from shoots to seeds can be inefficient. Interestingly, in PSC-plants, the results give an indication of species-specific response to the translocation of plant-available Se to seeds. Selenium concentration in the seeds was relatively higher in faba bean and wheat than in white lupin (Table 3). The results further showed significant ($P \leq 0.01$) and positive correlation coefficients for macronutrients (N, P) and Se concentration ($r = 0.925$, $r = 0.861$ respectively) in the grains of wheat. But, only seed P concentration positively correlated with Se concentration ($r = 0.860$, $P \leq 0.01$) in faba bean (Table 8). Although the reasons for the relatively high Se concentration in faba bean and wheat plants treated with PSC remains unclear, it is possible that wheat grains and faba bean seeds may be strong sinks for organic Se forms. Further studies are needed to fully understand the uptake and assimilation of organic Se in these plants.

The addition of NPK and PSC increased seed micronutrients (Fe, Zn and Cu) concentration (Table 7) above the baseline sufficiency level for Zn ($10\text{--}150\ \text{mg kg}^{-1}$), Fe ($20\text{--}300\ \text{mg kg}^{-1}$) and Cu ($5\text{--}30\ \text{mg kg}^{-1}$) (Graham et al. 2007; White and Broadley 2009). However, due to the relatively high trace elements concentration in pig slurry, micronutrients use efficiency (ZnUE, FeUE and CuUE) values were significantly lower in PSC-plants than those in NPK and control plants (Table 6). Interestingly, in all treatments, faba bean and wheat showed higher use (UE) efficiency values for Fe, Zn and Cu than those in white lupin. The reasons for species-specific responses cannot be explained in this study. However, the mechanisms involved in nutrients uptake are complex and also influenced by source/sink strength of each species. The results further showed significant ($P \leq 0.01$) and positive correlation coefficients for N and P concentration in seeds and Fe ($r = 0.857$, $r = 0.59$), Zn ($r = 0.413$, $r = 0.872$) and Cu ($r = 0.627$, 0.960) respectively in faba bean. Similarly, in wheat, grain N and P concentrations positively correlated with Fe, Zn and Cu (Table 8). This is consistent with the earlier findings in wheat (Cakmak, Pfeiffer, and McClafferty 2010) and faba bean (Baloch et al. 2014). These interactions suggest similar mechanisms or transporters may be involved in the uptake and translocation of these nutrients. Overall, the study shows pig slurry compost with half-dose of inorganic (NPK) fertilizer as a promising alternative to chemical fertilizers for improved micronutrients concentration in plants.

Conclusion

The present study provides indication on the positive effects of pig slurry compost with half-dose of NPK fertilizer (PSC) on micronutrients (Se, Fe, Zn and Cu) concentrations and use efficiency in *Vicia faba* L., *Lupinus albus* L. and *Triticum aestivum* L. The results showed that inorganic N (NPK) and PSC enhanced growth and micronutrients concentration in these plants as indicated by the significant increases in shoot and root biomass, seed yield, micronutrients concentration in shoots (leaves and stems) and seeds. The addition of Se-enriched NPK significantly increased Se use efficiency (SeUE), as well as, seed Se concentration to target levels. In contrast, PSC slightly elevated seed Se concentration and use efficiency (SeUE) in faba bean and wheat, but no such effect was observed in white lupin. Little is known about the cycling and availability of micronutrients in the manure-soil-plant system, hence the need for more studies to fully understand the mechanisms involved in the uptake of organic-bound micronutrients. In this study, PSC had positive effects on seed micronutrients (Zn, Fe, and Cu) concentration to sufficient levels in plants. But, micronutrients use efficiency (ZnUE, FeUE and CuUE) values were relatively lower in PSC-plants than NPK-plants. Further correlation analysis also showed that seed N and P concentrations were highly associated with seed micronutrients (Fe, Zn, Cu and Se) concentration. Overall, the results indicate that pig slurry compost with half-dose of NPK fertilizer is a promising alternative to chemical fertilizers for improved biomass and micronutrients (Fe, Zn and Cu) concentration in plants.

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Disclosure statement

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